

		Elongation		
5'DIG	-G-CAT-GGA-TCC-CAC-TGC-CCA-GGG-3'O	- Prime	r	
3′-	C-GTA-CCT-AGG-GTG-ACG-GGT-CCC	- CCC - CCG - CCC - CCA	Nuc	T1
3′-	C-GTA-CCT-AGG-GTG-ACG-GGT-CCC	- CCC - CCT - CCC - CCA	Nuc	T2
3′-	C-GTA-CCT-AGG-GTG-ACG-GGT-CCC	-CCC-CCA-CCC-CCA	Nuc	Т3
3′-	C-GTA-CCT-AGG-GTG-ACG-GGT-CCC	-AAA-ATC-AAA-ATA	Nuc	T4
3′-	C-GTA-CCT-AGG-GTG-ACG-GGT-CCC	- TTT - TAG - TTT - GTG	Nuc	T5
3′-	C-GTA-CCT-AGG-GTG-ACG-GGT-CCC	-GGG-GTC-GGG-CTC	Nuc	T 6
3′-	C-GTA-CCT-AGG-GTG-ACG-GGT-CCC	-TTT-TTT-CTT-GCT	Nuc	T 7
3′-	C-GTA-CCT-AGG-GTG-ACG-GGT-CCC	-TTT-CCC-TCT-GGG	Nuc	T8
3′-	C-GTA-CCT-AGG-GTG-ACG-GGT-CCC	-GGG-AAA-AGT-TTT	Nuc	T9
3′-	C-GTA-CCT-AGG-GTG-ACG-GGT-CCC	-CCC-CCA-CCC-CCT	Nuc	T10
3′-	C-GTA-CCT-AGG-GTG-ACG-GGT-CCC	-CCC-CCG-CCC-CCA	Nuc	T11
3′-	C-GTA-CCT-AGG-GTG-ACG-GGT-CCC	-GGG-GGC-GGG-GGA	Nuc	T12
3′-	C-GTA-CCT-AGG-GTG-ACG-GGT-CCC	- AAA - AA'T - AAA - AAC	Nuc	T13
3′-	C-GTA-CCT-AGG-GTG-ACG-GGT-CCC	~TTT~TTA-TTT-TTA	Nuc	T14
	22 nucleotides	12 nucleotides		
3′-	C-GTA-CCT-AGG-GTG-ACG-GGT-CCC	- AAA - AAA - AAA - AAA - AAA	NUC	T 15
3′-	C-GTA-CCT-AGG-GTG-ACG-GGT-CCC	-000-000-000-000-000	NUC	T 16
3′-	C-GTA-CCT-AGG-GTG-ACG-GGT-CCC	- TTT - TTT - TTT - TTT - TTT	NUC	T 17
3′-	C-GTA-CCT-AGG-GTG-ACG-GGT-CCC	- GGG-GGG-GGG-GGG-GGG	NUC	T 18
	SEQ ID NO: 6	SEQ ID NOS: 7-24		
	22 nucleotides	18 nucleotides		

10/019850

Fig.1b: Cassettes 1-10 template/ primer systems:

GTTAAGATTCGCCTCTAGCGG	CGTA	CAS	1
GTTAAGATTCGCCTCTAGCGG	CGTA-GTAC	CAS	2
GTTAAGATTCGCCTCTAGCGG	CGTA-GTAC-TAGC	CAS	3
GTTAAGATTCGCCTCTAGCGG	CGTA-GTAC-TAGC-ATCG	CAS	4
GTTAAGATTCGCCTCTAGCGG	CGTA-GTAC-TAGC-ATCG-CATG	CAS	5
GTTAAGATTCGCCTCTAGCGG	CGTA-GTAC-TAGC-ATCG-CATG-CAGT	CAS	6
GTTAAGATTCGCCTCTAGCGG	CGTA-GTAC-TAGC-ATCG-CATG-CAGT-TGCA	CAS	7
GTTAAGATTCGCCTCTAGCGG	CGTA-GTAC-TAGC-ATCG-CATG-CAGT-TGCA-TGAC	CAS	8
GTTAAGATTCGCCTCTAGCGG	CGTA-GTAC-TAGC-ATCG-CATG-CAGT-TGCA-TGAC-CAGT	CAS	9
SEO ID NO: 25			
GTTAAGATTCGCCTCTAGCGG	CGTA-GTAC-TAGC-ATCG-CATG-CAGT-TGCA-TGAC-CAGT-GATC	CAS	10
111111111111111111111			
AATTCTAAGCGGAGATCGCC-3′	Primer-5'DIG		
SEQ ID. NO: 26			
L	· Elongation		

Fig. 2

1 3 5 7 9 11 13 15 1719 21 23 25 27 29 31 33 Lane 2 4 6 8 10 12 14 16 18 20 22 24 26 28 30 32 34 number

Taq-I	Pol	Tgo exo polymerase									
0,1 ι	0,1 u		0,1u		u	0.1u 1u		0,1u	1 u	۱,	
T 17	T 16	T 17	T 16	T 17	T 16	T 8		T 3		1	
natural dNTP's		AA	BB	AA	BB	AAA AAA BB BB C C	AAAAAA BB BB C C	B B D	BB D	вввв EEFF]

Polymerase unit/ reaction Template (T)

Derivative

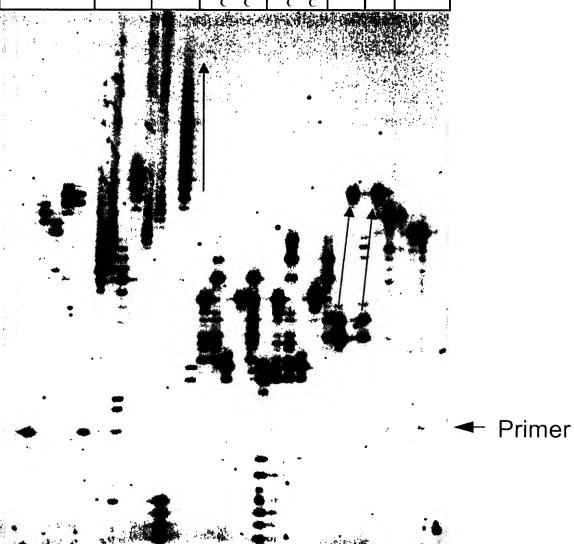
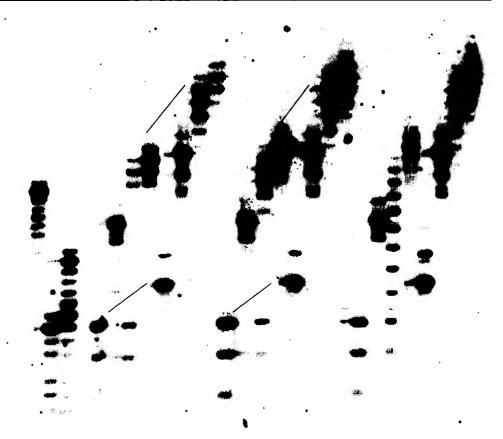


Fig. 3

1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 2 4 6 8 10 12 14 16 18 20 22 24 26 28 30

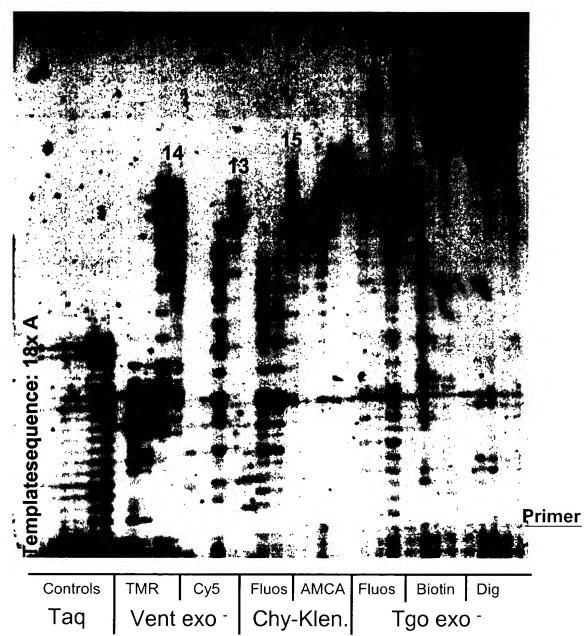
_	_												
Controls Tag Tgo exo polymerase						Tgo exoʻ polymerase							
pol	0	.1 unit/	1 unit/reaction										
	T4 T15 T4 T15			T4	T15	T4	T15	T 4	T 15	T 4	T 15		
	Reaction time: 30 m						nin 60 min						
regular	regular Rho-green- Rho-						Rho- Rho-			Rho-		Rho-	
dNTP's	dl	dUTP gre			green-		green-		green-		green- X-		
			X-d	UTP	dUTP		X-dUTP		dUTP		dUTP		



- Primer

Fig. 4

1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31 33
2 4 6 8 10 12 14 16 18 20 22 24 26 28 30 32 34



10/01/050

Fig. 5

1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31 33 35 2 4 6 8 10 12 14 16 18 20 22 24 26 28 30 32 34



Primer

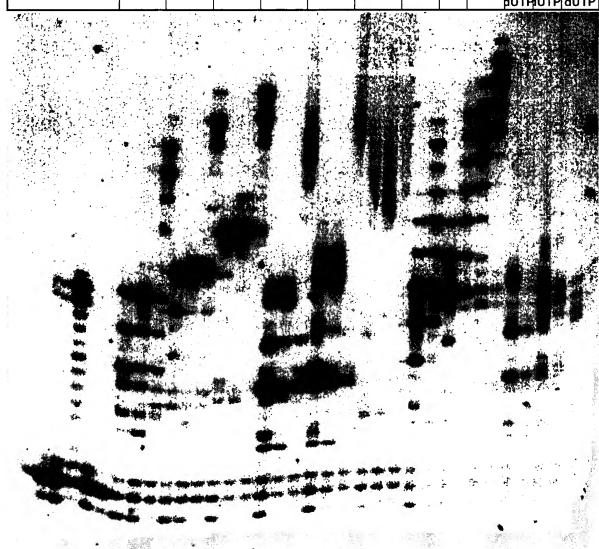
Control Taq

Taq polymerase

Chy-Klenow-fragment

1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31 33 35 2 4 6 8 10 12 14 16 18 20 22 24 26 28 30 32 34

Taq-pol control	<i>Tgo</i> exo⁻ pol											
	Template T4 Template T15											
natural dNTP's	Cy5- 17- dUTP	Cy5- 24- dUTP	Cy5- 38- dUTP	MR121- 8- dUTP	MR121- 13- dUTP	24-	Cy5- 17- dUTP	24-	38- dUTP	121- 8-	121- 13-	MR 121- 24- dUTP



Primer

Fig. 6a

11 13 15 17 1 2 3 4 5 6 7 8 9 10 12 14 16 18

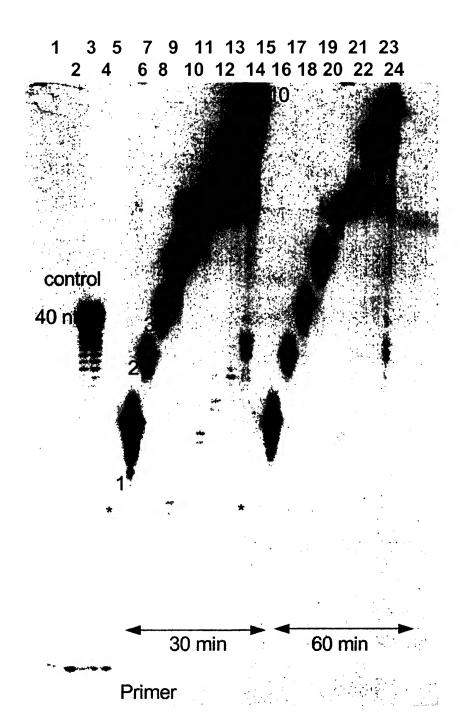


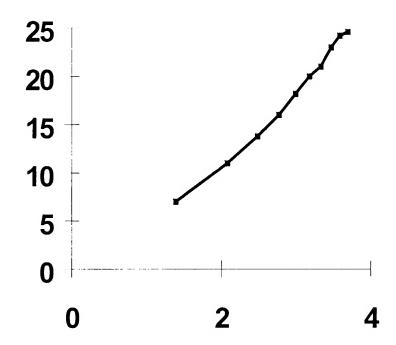
1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31 33 35 2 4 6 8 10 12 14 16 18 20 22 24 26 28 30 32 34 36

Taq-pol	Vent	Tgo	Vent	Tgo	Ve	ent	Tgo	Vent
0,1 unit		0,1	unit		0,5	unit	1u	0,5u
cass - 1 1 5 10 -	ca:	ss	С	ass 5	cass 5	40		cass 10
control	AAAA	AAAA	AAAA	AAAA	AAAA	AAAA	AAA	AAA
reactions	BBB	BBB	BBB	BBB	BBB	BBB	ВВ	EEE
natural	СС	СС	СС	СС	CC	СС	С	CC
dNTP's	D	D	D	D	D	D	D	D



Fig. 8





Ln of number of incorporated nucleotides

Fig. 9

Templateconcentration





60.010030

Formula of derivatives used:

MR-121-24-dUTP

MR-121-13-dUTP

MR-121-8-dUTP

Cy5-10-dUTP

Cy5-17-dUTP

Cy5-38-dUTP

Rhodamin-green-x-dUTP

Rhodamin-green-dUTP

Rosamin-dCTP

Dig-28-dCTP

7-Hexinyl-7-desaza-dATP ($Hex^7c^7A_d$)

7-Aminopentinyl-7-desaza-dATP

7-Hexinyl-7-desaza-dGTP ($Hex^7c^7G_d$)

List of nucleoside derivatives

d-adenosine and adenosine-analogue derivatives 7-deaza-dATP Digoxigenin-16-dATP Fluorescein-15-dATP Infrared₇₇₀-9-dATP 7-aminopentinyl-7-deaza-dATP N¹-[(acetamido)-3,6-dioxa-octylamino]-2'-deoxy-formycin A-triphosphate biotin-[N¹ (amidooctyl-3,6-dioxa-amidoacetyl)]-2'-deoxcy-formycin A-triphosphate d-formycin A-triphosphate N¹-methyl-formycin-A-triphosphate 7-hexinyl-7-deaza-dATP Digoxigenin-(7-aminohexinyl-7-deaza)-dATP TMR-(7-aminohexinyl-7-deaza)-dATP

d-cytidine- and cytidinanalogue derviatives

RhodaminGreen-(7-aminohexinyl-7-deaza)-dATP

Cy5-dCTP Digoxigenin-dCTP ψ-iso-dCTP Rosamin-dCTP

d-guanosine analogue derivatives

8-Aza-dGTP 8-Aza-7-bromo-7-deaza-dGTP 7-aminopentinyl-7-deaza-dGTP Cy5-(7-aminobutinyl-7-deaza)-dGTP 7-hexinyl-7-deaza-dGTP Fluorescein-(7-aminohexinyl-7-deaza)-dGTP RhodaminGreen-(7-aminobutinyl-7-deaza)-dGTP

d-uridine and uridine analogue derviatives

Aminoallyl-dUTP AMCA-6-dUTP Biotin-16-dUTP Bodipy-dUTP Carboranyl-dUTP Cy5-dUTP (with 10, 17, 24 and 38 atoms linker length) DÍG-11-dUTP Fluorescein-12-dUTP IF9-dUTP IRD40-dUTP JA53-dUTP IA133-dUTP JA218-dUTP MR121-dUTP (with 8, 13 and 24 atoms linker length) RN65-dUTP RhodaminGreen-dUTP (without 5 and with 12 atoms spacer length) ψ-dUTP Biotin-N¹-ψ-dUTP TMR-dUTP Estradiol-dUTP

Other derivatives

[Imidazo-(2-amino-s-Triazin-4-on)]-2'-deoxyribosid-triphosphate dITP Chinazolin-2'-deoxyribosid-triphosphate